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MAGNESIUM METABOLISM
Studies in health, primary hyperparathyroidism and renal stone disease.

by
GUNNAR JOHANSSON

Uppsala 1979

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From the Department of Internal Medicine,
University Hospital, Uppsala, Sweden

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UNIV.
LEIDEN
BIBL.

To Gunilla, Mikael, Stefan and Tomas

This thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Gastrointestinal magnesium absorption. Kinetic studies with ^{28}Mg and a simple method for determination of fractional absorption.
Bo G. Danielson, Gunnar Johansson, Bo Jung, Sverker Ljunghall, Hans Lundqvist & Petter Malmborg.
Mineral and Electrolyte Metabolism 2, 1978.

II. Magnesium metabolism in healthy subjects.
Bo G. Danielson, Gunnar Johansson & Sverker Ljunghall.
Scandinavian Journal of Urology and Nephrology, Suppl. 51, 49, 1979.

III. Magnesium homeostasis in primary hyperparathyroidism.
Bo G. Danielson, Gunnar Johansson & Sverker Ljunghall.
Submitted for publication.

IV. Magnesium metabolism in renal stone disease.
Ulla Backman, Bo G. Danielson, Gunnar Johansson, Sverker Ljunghall & Björn Wikström.
Submitted for publication.

V. Biochemical and clinical effects of the prophylactic treatment of renal calcium stones with magnesium hydroxide.
Ulla Backman, Bo G. Danielson, Bengt Fellström, Gunnar Johansson, Sverker Ljunghall & Björn Wikström.
Submitted for publication.

Conversion from traditional units to SI-units: 1 mmol Mg = 24.3 mg Mg.

CONTENTS

INTRODUCTION 6

The role of magnesium in biochemical reactions 6
Body stores 6
Serum magnesium concentration 6
Dietary intake and intestinal absorption 6
Renal excretion 7
Hormonal regulation of magnesium homeostasis 8
Magnesium deficiency 10
Renal stone disease 12

AIMS OF THE STUDIES 15

SUBJECTS 16

METHODS 16

RESULTS AND DISCUSSION 17

Intestinal absorption of magnesium 17
Renal magnesium excretion 20
Serum magnesium concentration 23
Muscle magnesium 23
Magnesium loading and retention 25
Correlation between magnesium parameters 25
Magnesium deficiency in HPT or renal stone disease ? 28
Magnesium metabolism in primary hyperparathyroidism 28
Magnesium metabolism in renal stone disease 29
Prophylactic treatment with magnesium of renal stone disease 30

GENERAL SUMMARY 33

ACKNOWLEDGEMENTS 35

REFERENCES 37

INTRODUCTION

The role of magnesium in biochemical reactions

Magnesium is a metal of general importance in the body and is involved in numerous biochemical reactions. It has an essential, regulatory function within the cell and activates or is at least essential for oxidative phosphorylation, all ATP-requiring reactions and both the nuclear transcription and the ribosomal translation in gene expression. Moreover, it is an activator of many enzymes, for instance, alkaline phosphatases, acid phosphatases, ATP-ases and pyrophosphatases (Aikawa, 1976).

Body stores

The adult human body contains about 25 g, i.e. approximately 1,000 mmoles of magnesium (Widdowson, McCance & Spray, 1951). It is the fourth most abundant cation in the body and after potassium the most plentiful intracellularly (Wacker & Parisi, 1968). Bone contains about 60 per cent of the total magnesium in the body. Most of the remainder is almost equally distributed between muscle and non-muscular, soft tissues (Aikawa, 1976).

Serum magnesium concentration

The serum magnesium concentration, which accounts for less than 1 per cent of the total body magnesium, is generally maintained within narrow limits, with a range of 0.7 - 1.0 mmol/l (Briscoe & Ragan, 1967; Jackson & Meier, 1968; Keating, Jones, Elveback & Randall, 1969; Christiansen & Transbøl, 1978). One-third of the serum magnesium is protein-bound (most of it to albumin), while the rest is present in a diffusible form (Massry & Seelig, 1977; Danielson, Pallin & Sothell, 1979). Absorption of magnesium from the gut and its excretion by the kidneys are the main factors determining the serum magnesium levels (McIntyre & Robinson, 1969).

Dietary intake and intestinal absorption

The average daily intake ranges between 10 and 20 mmoles of magnesium, and an intake of 0.15 mmoles per kilogram of body weight per day is reported to

be required to maintain a positive magnesium balance (Jones, Manalo & Flink, 1967). There is a linear relation between the dietary magnesium and the amount absorbed over a wide range of intakes. In other words, the fractional, intestinal absorption is constant within the normal range of dietary magnesium and in balance studies has been assessed as about one--third of the intestinal load (Graham, Caesar & Burgen, 1960; King & Stanbury, 1970). The absorption varies with the dietary intake of other nutrients, for instance, proteins, carbohydrates, calcium and anions, particularly phosphate (Wilkinson, 1976). The details of magnesium absorption are largely unknown. A saturable, facilitated, diffusion process, located proximally in the small intestine, has been proposed, probably partly regulated by the vitamin D system (Brannan, Vergne-Marini, Pak, Hull & Fordtran, 1976; Wilkinson, 1976; Levine, Brautbar, Lee, Walling, Kurokawa, Kleeman & Coburn, 1979), together with a more ion-specific process located distally in the small intestine and probably independent of the action of vitamin D (Wilkinson, 1976).

Renal excretion

The diffusible fraction of blood magnesium is filtered by the glomerulus in an amount of about 100 mmoles per 24 hours. Three to five per cent of the filtered magnesium is excreted in the final urine, whereas the rest is reabsorbed, mainly in the ascending part of the distal tubule (Dirks & Quamme, 1978). This reabsorptive mechanism normally operates close to its maximum (Chesley & Tepper, 1958; Massry & Coburn, 1973), although, in a state of magnesium-deficiency the renal conservation of magnesium is pronounced (Shils, 1969). At least in rats, a tubular secretion of magnesium seems to occur under conditions of magnesium-loading, i.e. when the serum level exceeds a critical value, close to the upper limit of the normal range (Heaton, 1969). It has not been established whether there is also a tubular secretion in human beings.

Hormonal regulation of magnesium homeostasis

Parathyroid hormone

Evidence is accumulating that the parathyroid hormone (PTH) is involved in the regulation of magnesium homeostasis. In balance studies of patients suffering from primary hyperparathyroidism (HPT), the intestinal absorption of magnesium is increased and returns to normal after surgery (Heaton & Pyrah, 1963; King & Stanbury, 1970). However, the net magnesium balance in patients with HPT has been reported to be slightly negative prior to surgery (Hanna, North, MacIntyre & Fraser, 1961; Wacker & Parisi, 1968; King & Stanbury, 1970), due to an increased, renal excretion. This raised, urinary magnesium output in cases of HPT still occurs, even though PTH per se increases the magnesium reabsorption in the renal tubules (MacIntyre, Boss & Troughton, 1963; Eliel, Smith, Chanes & Hawrylko,1969; Massry & Seelig, 1977) and is likely to be the result of interference between the renal handling of magnesium and calcium. Thus, since magnesium and calcium share a common transport system in the kidneys, the increased, filtered load of calcium inhibits the tubular reabsorption of magnesium, which, in turn, overcomes any direct effect of PTH on the tubular handling of magnesium (Hornum & Transbøl, 1973; Massry & Coburn, 1973).

In cases of parathyroid insufficiency, it is suggested that the intestinal absorption of magnesium is reduced, the retention of intravenously administered magnesium is increased, and the serum magnesium concentration may be below normal (Jones & Fourman, 1966), thus indicating a magnesium deficiency.

On the other hand, magnesium appears to be essential for the secretion of PTH (Sherwood, Hanley, Takatsuki, Birnbaumer, Schneider & Wells, 1977) and in cases of severe magnesium deficiency a state of functional hypoparathyroidism may ensue, due to both a reduced PTH release from the parathyroid glands (Anast, Winnacker, Forte & Burns, 1976; Rude, Oldham, Sharp & Singer, 1978) and an impaired peripheral response to the hormone (Ashby & Heaton, 1975; Rude et al., 1978).

Vitamin D

In the rat, the administration of vitamin D (Wasserman, 1962) or several of the vitamin D metabolites (Levine et al., 1979) increases the intestinal absorption of magnesium. In man, conflicting results of the action of vitamin D on the intestinal handling of magnesium have been reported. Both an increased uptake (Revusova, Gratzlova, Zvara & Dzurik, 1972; Brannan et al., 1976; Sörensen, Tougaard & Bröchner-Mortensen, 1976) and an absence of effects on the intestinal absorption (Coburn, Brickman, Hartenbower & Norman, 1976) are reported.

Calcitonin

The administration of calcitonin to human beings has been associated with an increased (Paillard, Ardaillou, Malendin, Fillastre & Prier, 1972) or an unchanged urinary magnesium excretion (Cochran, Peacock, Sachs & Nordin, 1970). The effect on the intestinal absorption in human beings is unknown, but the hormone has been found to decrease the absorption in sheep (Care & Keynes, 1964).

Magnesium and other hormones

Other hormones might be involved in magnesium homeostasis, for instance, thyroid hormone and aldosterone. Excess of either hormone causes a negative magnesium balance by decreasing the intestinal absorption and increasing the urinary excretion of magnesium (Wacker & Parisi, 1968; Wallach, 1976). Also corticosteroids, growth hormone, insulin and glucagon are reported to affect the magnesium metabolism (Wacker & Parisi, 1968).

Relation between magnesium and calcium

The magnesium and calcium homeostases appear to be regulated, at least in part, by common mechanisms. The two ions share transport systems in the gut (Alcock & MacIntyre, 1962; Heaton, Hodgkinson & Rose, 1964; Clarkson, Warren, MacDonald & de Wardener, 1967) and the kidneys (Massry & Coburn, 1973) and are affected in a similar way by PTH (Wilkinson, 1976) and vitamin D (Levine et al., 1979).

A magnesium-regulating hormone?

There is probably a very complex system for maintaining the intracellular magnesium homeostasis, since a marked fall in serum magnesium, due to magnesium deficiency, is often associated with nearly normal tissue and bone magnesium content (Rasmussen & Bordier, 1974). Oreoupoulos and his colleagues (1969) also asserted the existence of a homeostatic system for regulating the extracellular magnesium metabolism. Some kind of regulation of the serum magnesium levels is likely to be present, since the serum values are remarkably constant within the individual and also between different groups of patients with mineral, metabolic disturbances (Nordin, 1976).

However, there is at present no convincing evidence to support the hypothesis of the existence of a specific, magnesium-regulating hormone, but the regulation of magnesium homeostasis is considered to be the result of a number of hormonal and non-hormonal actions, in which the calciotropic hormones seem to be of predominant importance.

Magnesium deficiency

A selection of the causes of magnesium deficiency and hypomagnesemia is given in Table I. Details concerning the etiology and clinical picture have been excellently reviewed, for example, by Flink (1976).

The serum magnesium concentration is not always a reliable guide to the total magnesium balance, since a large deficit can occur without concomitant hypomagnesemia (Massry & Seelig, 1977). Nor is the renal excretion of magnesium a reliable index of the magnesium balance, because an extremely low output may only indicate a corresponding, low, dietary intake and not reflect the total balance. Bone and muscle contain most of the magnesium in the body (Aikawa, 1976). Isotope studies have demonstrated that the muscle magnesium is a readily accessible reservoir buffering losses of magnesium from the body, whereas bone magnesium is affected only when a state of prolonged, magnesium depletion is apparent (Lim, Jacob, Dong & Khoo, 1969). The relative ease of obtaining a muscle specimen, compared with bone biopsies, and the convenient method of estimating intracellular, muscle magnesium has

Table I. Some causes of magnesium deficiency and hypomagnesemia, modified after Flink (1976).

I.	Decreased, magnesium intake
	Prolonged, intravenous therapy without additional magnesium
	Chronic alcoholism
	Starvation
II.	Decreased, intestinal absorption
	Malabsorption due to surgical resection
	Severe diarrhoea, e.g. ulcerative colitis, regional enteritis, laxative abuse, coeliac disease
	Pancreatitis
III.	Excessive renal excretion
	Diuretics
	Hypercalciuria
	Hyperaldosteronism
	Diabetic ketoacidosis
	Chronic alcoholism
	Renal tubular acidosis
	Familial, renal magnesium wastage
	Diuretic phase after acute, tubular necrosis

also made this a suitable method for clinical purposes (Bergström, 1975). Alfrey et al. (1974), however, suggested that bone magnesium may yield a more reliable estimate and may correlate better with serum magnesium than muscle magnesium.

Estimations of the magnesium content of erythrocytes, lymphocytes, hair and nails have also been used, but further clinical evaluation of these methods is necessary (Flink, 1976).

Magnesium-infusion tests are quite easily performed and have been used for the diagnosis of deficiency (Caddell, Ratananon & Trangratapit, 1973; Caddell, Saier & Thomason, 1975). The amount of magnesium excreted in the urine after the load is believed to give an estimate of the amount that is retai-

ned in the body. Aikawa (1976) demonstrated a high degree of retention in healthy subjects, when the loading amount was given together with a tracer, ^{28}Mg, but a simultaneous, urinary excretion of stable magnesium in an amount equal to that given, due to excretion of magnesium from other body stores. An increased retention has been demonstrated in some studies in patients with proposed magnesium deficiency (Thorén, 1963; Jones & Fourman, 1966).

Renal stone disease

Physiochemical aspects

Most stones formed in the kidneys are composed of calcium oxalate, calcium phosphate or a mixture of both. There are two main mechanisms that are of probable importance in the formation of renal calcium stones (Fleisch, 1978):

1) The concentration of precipitating substances, i.e. mainly calcium, oxalate and phosphate, and the solubility of the mineral phase formed and

2) The inhibitory activity protecting against the precipitation and aggregation of these calcium salts.

The urine from most stone-formers is supersaturated with respect to calcium oxalate or calcium phosphate. As the degree of supersaturation varies and as there is a great overlap between stone formers and non-stone-forming subjects, much attention has been directed to distinguishing between these two groups. Subjects without renal stone disease excrete no or only individual crystals in the urine, whereas stone-formers excrete greater amounts of crystals and, in addition, tend to excrete large aggregations of crystals (Robertson & Peacock, 1972). The main inhibitors of the precipitation of calcium oxalate and calcium phosphate crystals are citrate, pyrophosphate and magnesium, whereas glucosaminoglucans, besides citrate and pyrophosphate, inhibit the aggregation of formed crystals in the urine (Fleisch, 1978).

The pathogenesis of renal stones: A role for magnesium ?

Hammarsten (1929) indicated in an early study that magnesium can increase the solubility of calcium oxalate *in vitro*. Others (Gershoff & Andrus, 1961; Borden & Lyon, 1969; Schneider, Hesse, Berg, Kirsten & Nickel, 1977) have shown that magnesium decreases the incidence of experimental, calcium oxalate stone formation. Desmars & Tawashi (1973) emphasized the fine kinetic control which magnesium exerts on calcium oxalate formation, because of the greater solubility product for magnesium oxalate than for calcium oxalate. Mukai & Howard (1963) coined the terms "evil" and "good" urine, where the "evil" urine from stone formers was found to calcify rachitic-rat cartilage, while normal, "good" urine did not. The addition of magnesium to calcifying urine changed it to non-calcifying urine. Using a quantitative technique to determine the inhibitors of calcium phosphate precipitation, magnesium was found to account for 20 per cent of the total inhibitory activity (Bisaz, Felix, Neuman & Fleisch, 1978). However, other authors have been unable to confirm this inhibitory propensity of magnesium, but these studies were undertaken on the urine from normal subjects without stone disease (Elliot & Ribeiro, 1973; Meyer & Smith, 1975) or on just a few stone-formers during a short observation period (Elliot & Ribeiro, 1971; Fetner, Barilla, Townsend & Pak, 1978).

Another possible role of magnesium in cases of stone disease has been suggested on the basis of animal experiments. Young rats fed on a magnesium--deficient diet rapidly developed renal calcification (Bunce & King, 1978), in which the majority of calcium was present as apatite crystals in the proximal, tubular cells (Battifora, Eisenstein, Laing & McCreary, 1966). This phenomenon can also be produced with a vitamin B_6-deficient diet in rats, whereas a diet containing abundant magnesium protects against stone formation (Faragalla & Gershoff, 1963). However, whether this experimentally obtained, stone formation during magnesium deficiency is comparable to human stone disease remains to be elucidated.

Magnesium in renal stone disease: Clinical observations

As a low, urinary excretion of magnesium may, from a physicochemical viewpoint, be expected to be associated with a reduced, inhibitory activity and therefore an increased, stone-forming diathesis, the urinary magnesium output has been determined in a number of clinical studies. In general, stone formers appear to have a magnesium excretion not different from that obtained in healthy, non-stone-forming, control subjects (Takasaki & Shimano, 1967; Evans, Forbes, Sutton & Watson, 1967; Takasaki, 1972; Raman & Sreenevasan, 1972; Jörgensen, 1975; Welshman & McGeown, 1975; Ljunghall & Waern, 1977; Zechner & Latal, 1978). In some studies, however, stone formers were reported to have a low, urinary magnesium excretion, either in comparison with controls not matched for age and sex (Hodgkinson, 1974; Matouschek, Huber, Schneider & Vogg, 1978), or in comparison with an adequate matched control group (Schwille, Schlenk, Samberger & Bornhof, 1976; Tiselius, Almgård, Larsson & Sörbo, 1978).

As described earlier, the urinary excretions of magnesium and calcium are positively correlated, so that, for increasing values of urinary calcium, the urinary magnesium rises. Most stone formers have raised values of urinary calcium, but, in relation to the calcium excretion, the magnesium output appears to be inappropriately low, i.e. the magnesium excretion for corresponding calcium values is in general lower in the stone-forming patients (Ljunghall & Waern, 1977). The ratio of urinary magnesium to urinary calcium has been used as an index of the stone-forming propensity, and a low value has been reported to be associated with an increased frequency of stones (Oreopoulos, Soyannwo & McGeown, 1968; King, O'Connor, Smith & Crouse, 1968; Takasaki, 1972; Scott, Sinclair, Fell & Wilson, 1974; Hodgkinson, 1974; Jörgensen, 1975; Welshman & McGeown, 1975; Schwille et al., 1976; Ljunghall & Waern, 1977; Tiselius et al., 1978). Further, in epidemiological studies, it has been demonstrated that, irrespective of the level of urinary calcium, a high ratio of urinary magnesium to urinary calcium is associated with a lower prevalence of stones (Ljunghall & Waern, 1977). Thus, although the total urinary magnesium in stone formers often is not significantly different from that obtained in control subjects, there is evidence that magnesium plays a protective role in renal stone disease.

Determinations of the serum and urinary magnesium concentrations have not suggested that magnesium deficiency is a feature in renal stone disease, but more detailed studies of the magnesium homeostasis have not been performed in patients with renal stone formation.

Magnesium has been used in clinical trials as a prophylactic treatment against recurrent, renal stone formation (Ljunggren, 1965; Albuquerque & Tuma, 1962; Moore & Bunce, 1964; Kohler & Uhle, 1966; Gershoff & Prien, 1967; Melnick, Landes, Hoffman & Burch, 1971; Silver & Brendler, 1971; Prien & Gershoff, 1974; Terhorst & Jonas, 1974; Fetner et al., 1978; Joost & Purtscheller, 1978). The available data indicate that the recurrence rate is significantly reduced during the treatment, but unfortunately only one study (Melnick et al., 1971) reports on the natural history in untreated, control subjects. The number of participants has often been few and also the number of drop-outs, in whom the effects of treatment are unknown, has been comparatively high. Therefore, it is difficult to draw valid conclusions as to the possible beneficial effect of magnesium therapy on stone recurrence from these studies. Adverse effects of the therapy appear to be uncommon, but the possible side effects of long-term therapy are not evaluated.

AIMS OF THE STUDIES

The present studies were undertaken in order to investigate the magnesium metabolism in health and in cases of primary hyperparathyroidism and renal stone disease and to evaluate the therapeutic effect of magnesium on stone recurrence.

The aims of the studies were as follows:

- To develop a technique for measuring the kinetics and the fractional absorption in the intestine of the short-lived radionuclide ^{28}Mg and to apply this technique to healthy subjects and to patients with primary hyperparathyroidism or renal stone disease (I, II, III, IV).

- To evaluate the magnesium data in healthy subjects, in order to obtain a frame of reference (II).

- To study the magnesium metabolism in cases of primary hyperparathyroidism, with particular attention to stone formation, and to evaluate the effects of parathyroidectomy (III).

- To assess the magnesium metabolism in patients with renal stone disease and to evaluate whether magnesium deficiency is a feature of renal stone formation (IV).

- To assess the value of prophylactic treatment with magnesium hydroxide in cases of renal stone disease (V).

SUBJECTS

1. Apparently healthy subjects (II) recruited from a general health survey formed a control group, together with medical students (II) and members of the staff (I, II).

2. Altogether 28 patients with surgically proven, primary hyperparathyroidism were investigated prior to and after neck surgery (III).

3. Seventy consecutive patients admitted with renal stone formation were studied with regard to the magnesium metabolism (IV).

4. From the previous group, 56 patients were started on prophylactic, magnesium therapy (V).

METHODS

Besides routine, clinical investigation, a special programme was implemented, in order to evaluate the magnesium metabolism. This included measurements of the serum magnesium, the urinary magnesium, the intracellular magnesium in muscle specimens obtained by percutaneous biopsies, the fractional, intestinal absorption, utilizing the radionuclide ^{28}Mg, an intravenously administered magnesium-loading test and the calculation of the magnesium retention on the basis of the results of this loading test.

RESULTS AND DISCUSSION

Intestinal absorption of magnesium (I, II, III, IV)

The intestinal absorption of magnesium was assessed by a tracer technique, utilizing the radionuclide ^{28}Mg. External, photon detectors were used to measure the uptake in the forearm after both intravenous and oral administration of the isotope, usually at an interval of a week. The rate of absorption after oral ingestion was constant during the first five hours, except for the first 0.5 hours (I). Similar values were obtained by Graham et al. (1960), by measuring the activity in the blood after oral administration. Thus, if the site of absorption is inferred only from the time when radioactivity appears in the plasma, the data are consistent with a fairly uniform absorption throughout the small intestine (I). Brannan et al. (1976) obtained similar results, utilizing a local, perfusion technique in the human intestine. Chutkow (1964), however, suggested that the main part was absorbed in the large bowel, at least in rats; that a colonic absorption of magnesium may occur also in human beings, under certain circumstances, is evidenced by the hypermagnesemia developing after rectal enemas (Stevens & Wolff, 1950).

The technique also yielded a measurement of the true absorption of ^{28}Mg. This is probably almost equal to the net absorption, as the endogenous secretion into the intestinal lumen is estimated to be only 1-2 per cent (Graham et al., 1960; Avioli & Berman, 1966). The fractional absorption was calculated as the ratio between the measurements at 24 and 20 h after the oral and intravenous administrations, respectively. The 4-hour shift in the measuring-point was an approximate correction for the delayed appearance of ^{28}Mg in the circulation after oral administration (I). This fractional absorption was, on the average, 42 $\pm$ 7 % (mean $\pm$ S.D.) in apparently healthy subjects (I, II), 46 $\pm$ 6 % in patients with primary hyperparathyroidism before neck exploration (III) and 41 $\pm$ 6 % in renal stone formers (IV) (Fig. 1). Conventional balance studies (Heaton & Pyrah, 1963; King & Stanbury, 1970) and a few tracer studies (Graham et al., 1960; Avioli & Berman, 1966; Schwartz, Spencer & Wentworth, 1978) have yielded similar results in healthy subjects. Six of the seven patients with HPT decreased their intestinal absorption of ^{28}Mg after parathyroidectomy, thereby obtaining a mean

value of 41 ± 7 % (± S.D.) (III), which is close to the value found in healthy subjects. Also Heaton & Pyrah (1963) and King & Stanbury (1970) demonstrated a postoperative decrease in magnesium absorption in balance studies of patients with HPT.

When the data from all the groups studied were pooled together, a slight reduction of the fractional, intestinal absorption of magnesium with age could be demonstrated (Fig. 2), similar to that earlier described for calcium (Bullamore, Gallagher, Wilkinson, Nordin & Marshall, 1970).

The isotope studies performed to assess the intestinal absorption of ^{47}Ca, on renal stone formers admitted to our stone clinic have previously been reported (Ljunghall, Backman, Danielson, Johansson, Jung, Källsen, Wibell & Wålinder, 1977). Most of these patients had an increased, intestinal absorption of calcium, indicating that this is a common cause of the hypercalci-

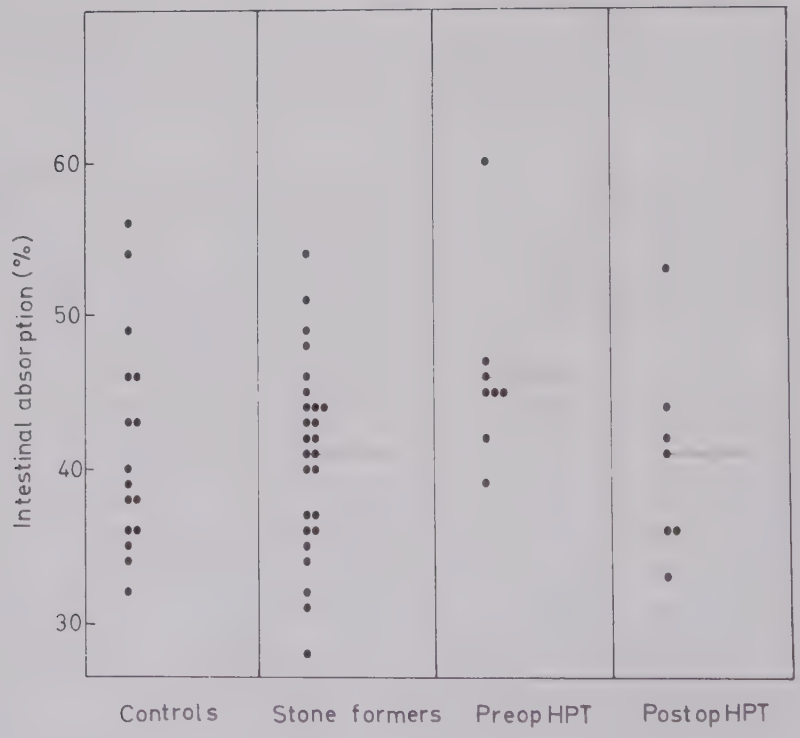

Fig. 1. The fractional, intestinal absorption in healthy subjects, renal stone formers and patients with HPT, before and after parathyroidectomy. The mean values are indicated by solid lines.

uria often seen in renal stone disease. Kinetic studies disclosed a rapid absorption of calcium after oral administration in agreement with Smart & Holdsworth (1978), suggesting an absorption mainly in the proximal part of the small intestine (Wilkinson, 1976). When data for the absorption of magnesium and calcium are compared, it seems likely that the intestinal uptake of the two ions is partly ion-specific.

However, it should be observed that the techniques for assessing the uptake and the amount of the stable ion given together with the isotope differed somewhat in our two studies. The differences in the absorption patterns between the two ions, could, however, also depend on the proposed accumulation of magnesium in the liver initially after absorption from the oral load (Aikawa, 1976), a phenomenon that has not been demonstrated for calcium.

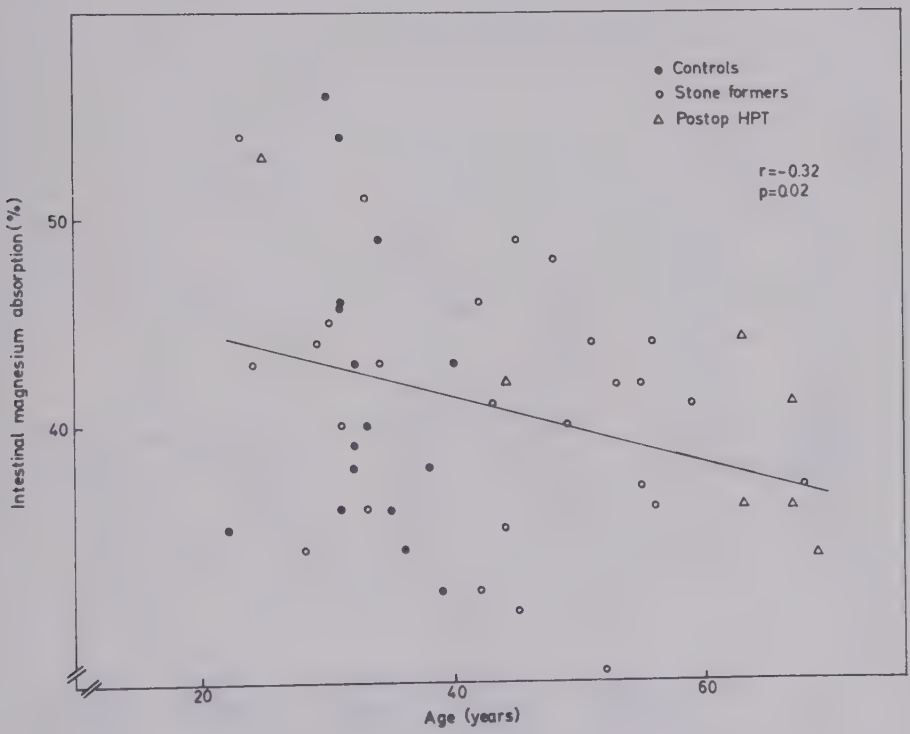

Fig. 2. The relation between the fractional, intestinal absorption and the age in healthy subjects, renal stone formers and patients with HPT after parathyroidectomy.

Renal magnesium excretion (II, III, IV, V)

Besides the intestinal absorption process, the renal excretion appears to be the main regulating mechanism involved in the magnesium homeostasis. The urinary output in healthy subjects and in patients with primary HPT and renal stone disease, compared with controls matched for age and sex, is illustrated in Fig. 3. The average urinary excretion of magnesium was found to be higher in healthy men than in healthy women at all ages and the excretion also decreased with age in both sexes (II). A correction for the simultaneously obtained, urinary creatinine excretion in the healthy subjects, i.e. a correction for the lean body mass (Muldowney, Crooks & Bluhm, 1957), however, eliminated this sex and age difference (II). The observed difference in output at different ages may be a reflection of the higher, fractional, intestinal absorption in younger individuals (Fig. 2) or due to differences in the dietary intake of magnesium.

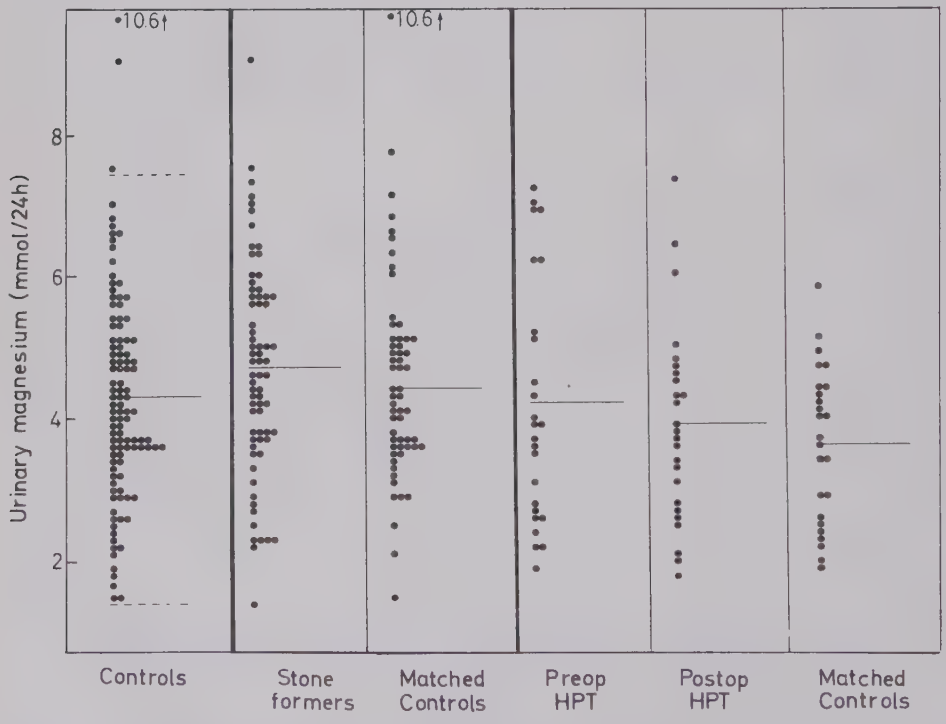

Fig. 3. The urinary excretion of magnesium in healthy subjects, renal stone formers and patients with HPT, before and after parathyroidectomy, in comparison with controls matched for age and sex. The solid lines indicate the mean values, whereas the dashed lines denote ± 2 S.D.

In agreement with previous reports (King et al., 1968; Sutton & Watson, 1969; King & Stanbury, 1970; Hodgkinson, 1974; Welshman & McGeown, 1975; Schwille et al., 1976; Ljunghall & Waern, 1977), all the studies showed a positive relationship between the urinary magnesium and urinary calcium excretions (II, III, IV). This relationship is probably due to a common reabsorption system in the kidney (Massry & Coburn, 1973), although calcium is mainly reabsorbed in the proximal tubule (Wilkinson, 1976) and magnesium in the ascending part of the distal tubule (Dirks & Quamme, 1978). A weak correlation was also found between the urinary excretion and the fractional, intestinal absorption of magnesium, although the isotope study and the urinary collections were performed under different dietary conditions ($\underline{r} = 0.44$; $p < 0.02$) (IV).

The increased, intestinal magnesium absorption and bone resorption pre--operatively in patients with HPT would be expected to be associated with an elevated, urinary magnesium output. This was also true, when HPT patients were compared with control subjects matched for age and sex (III) (Fig. 3). However, in comparison with the renal stone formers, who had an urinary excretion of calcium of the same magnitude, and thus presumably an equal increase of circulating, active, vitamin D metabolites (Kaplan, Haussler, Deftos, Bone & Pak, 1977), the patients with HPT had a lower magnesium output (Fig. 3). This difference may have been due to an increased tubular reabsorption of magnesium in HPT mediated by elevated levels of circulating PTH.

The patients with HPT reduced their magnesium outputs after parathyroidectomy (III) (Fig. 3), probably as an effect of the decreased, intestinal magnesium absorption and the reduced bone resorption of magnesium. The lowered, filtered load of calcium in the kidneys also tends to decrease the magnesium output. These effects of parathyroidectomy appear to predominate over the decreased, PTH-dependent, tubular reabsorption of magnesium.

The stone formers had a mean value of urinary magnesium similar to that obtained in the control subjects (Fig. 3) and male stone formers even had a slightly greater excretion than healthy male subjects (IV). However, in comparison with the healthy subjects, the stone-forming subjects had a

tendency to lower magnesium output for any given level of urinary calcium (Fig. 4).

The stone-forming subjects thus had a lower ratio of urinary magnesium to urinary calcium, mostly as a consequence of their greater urinary calcium output (IV). This ratio was correlated to the urinary calcium excretion in all groups (Fig. 5), whereas no such consistent correlation could be obtained for the quotient in relation to the urinary magnesium excretion. This absence of relationship was probably due to the narrow range of magnesium excretion in all groups (II, III, IV).

When the stone formers were given prophylactic, magnesium treatment, their urinary magnesium excretion increased, whereas the calcium output remained unchanged, and consequently the regression line between urinary magnesium and urinary calcium shifted (V) (Fig. 4). The ratio of urinary magnesium to

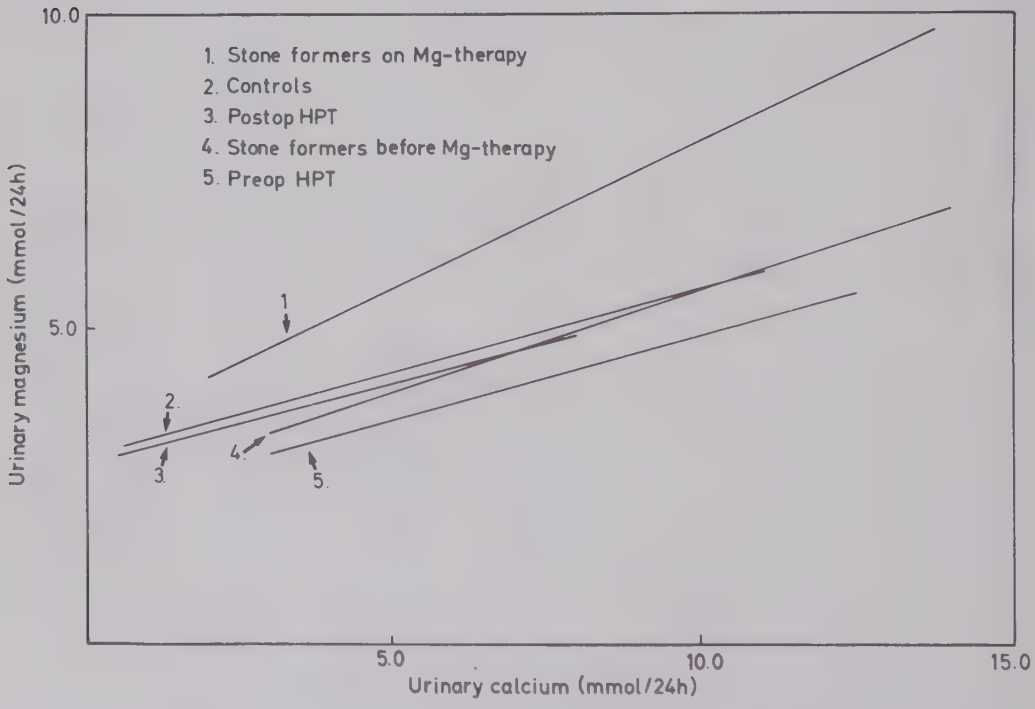

Fig. 4. The relation between the urinary excretion of magnesium and calcium in healthy subjects, renal stone formers and patients with HPT.

urinary calcium also increased on treatment and approached the value found in healthy subjects (V).

Serum magnesium concentration (II, III, IV, V)

The values of serum magnesium showed a narrow range in healthy subjects, as well as in HPT patients and renal stone formers (Fig. 6). No age and sex differences were observed in the different groups. These data compare favourably with those in previous reports (Briscoe & Ragan, 1967; Jackson & Meier, 1968; Keating et al., 1969; Christiansen & Transbøl, 1978). The serum magnesium was slightly increased during the first six months of magnesium therapy but thereafter decreased to pre-treatment level (V).

Muscle magnesium (II, III, IV, V)

The muscle magnesium was determined in specimens obtained by percutaneous biopsies from the lateral portion of the lateral quadriceps muscle. A

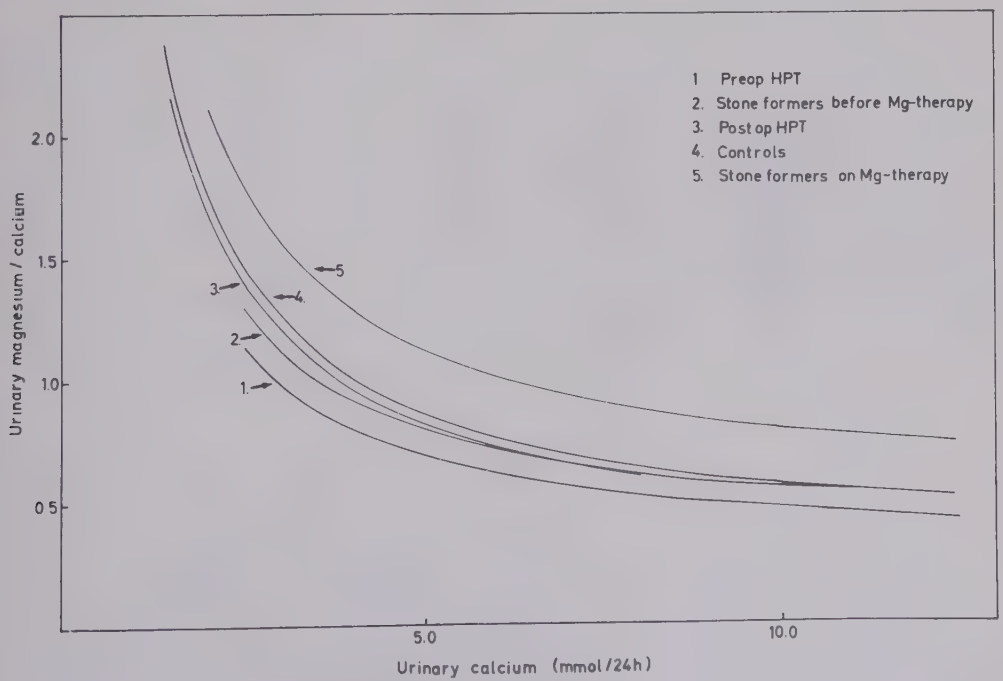

Fig. 5. The relation between the calcium and the urinary magnesium/calcium ratio in healthy subjects, renal stone formers and patients with HPT.

fat-free, solid biopsy was then obtained, after the fat had been extracted with petroleum ether and the specimen had been dried in an oven. The analysis was performed with atomic absorption.

No age and sex dependence was observed for the magnesium values in the muscle specimens. The analysis of biopsies from subjects with either renal stone disease (IV) or primary HPT (III) did not, display any low values. Biopsies were also taken from the patients with HPT after parathyroidectomy had been performed (III) and from some of the subjects with renal stones who had been on treatment with magnesium hydroxide for two years (V). There was no difference in magnesium content in these biopsies, in comparison with the first biopsy from the same patients taken at the first visit. Thus, utilizing this method, intracellular magnesium deficiency appeared to be uncommon in cases of both mild-to-moderate, primary HPT and renal calcium stone disease.

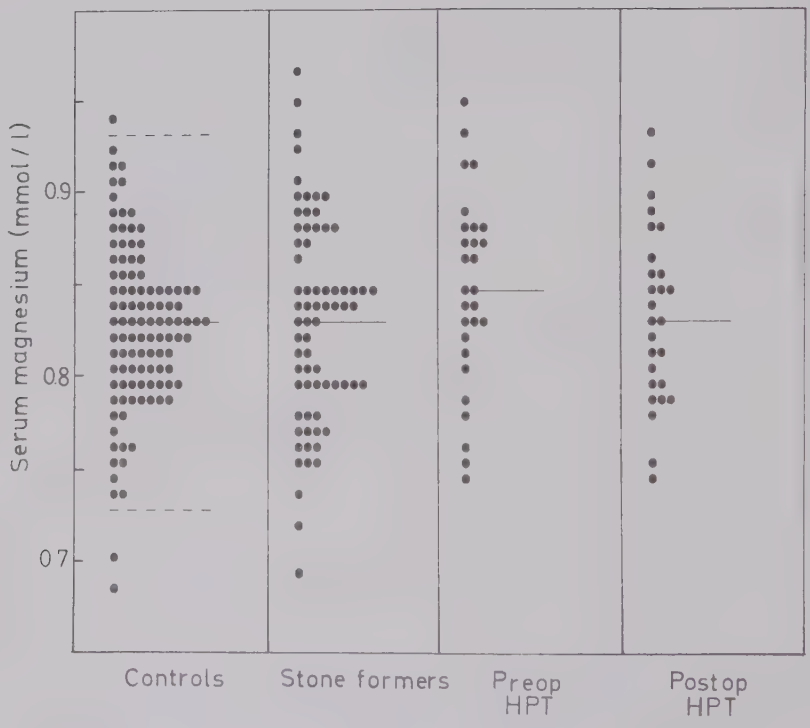

Fig. 6. The serum magnesium concentrations in healthy subjects, renal stone formers and patients with HPT, before and after parathyroidectomy. The solid lines indicate the mean values, whereas the dashed lines denote $\pm$ 2 S.D.

Magnesium loading and retention (II, III, IV)

A magnesium-loading test was performed, in which 10 mmoles of magnesium sulphate were given intravenously as an infusion in a carbohydrate solution. As the excreted, 24-hour amount of magnesium in the urine after this loading was found to be correlated with the basal, urinary output of magnesium (II), the patients' basal, urinary excretion had to be considered when calculating the retention of the load (II). One way of assessing this retention was simply to subtract the basal, urinary amount from the amount excreted after the loading and then to relate this value to the amount given. However, as the subjects were not restricted in their diet during the study period, some magnesium was also contributed by the intestinal absorption. As this absorbed amount could be approximately equal to the basal, urinary amount in individuals in magnesium balance, the total magnesium load was calculated as the sum of the intravenously given amount and the value obtained from the basal output of magnesium, The retention could then be determined as the difference between this total load and the amount excreted after the loading, expressed as a percentage of the total load.

This latter method of calculation, i.e. in relation to the total load, provided a more gaussian-like distribution of the values and the range was also considerably narrowed in the control group (II). The magnesium retention was found to be within the normal range in both renal stone formers (IV) and patients with HPT, with no change in retention after parathyroidectomy (III). If this is a reliable test for disclosing magnesium deficiency - the discovery, which was the initial purpose in designing the test (Thorén, 1963) - there was no evidence of magnesium deficiency in patients with HPT or renal stone disease (Table II).

Correlation between magnesium parameters (II, III, IV)

The correlations between different magnesium parameters were evaluated in healthy subjects, and in patients with HPT and renal stone disease, separately or together. A positive, although weak, correlation was suggested between the fractional, intestinal absorption of magnesium and the urinary excretion of magnesium ($r = 0.44$; $p < 0.02$) (IV), although there was no

Table II. Magnesium retention in healthy subjects, and in patients with primary hyperparathyroidism and renal stone disease.

		Mean ± SD (%)	Range
Healthy subjects	(n = 34)	20 ± 11	0 - 38
Primary HPT, preop.	(n = 17)	22 ± 13	0 - 39
Primary HPT, postop.	(n = 25)	19 ± 9	0 - 35
Renal stone disease	(n = 60)	21 ± 14	0 - 48

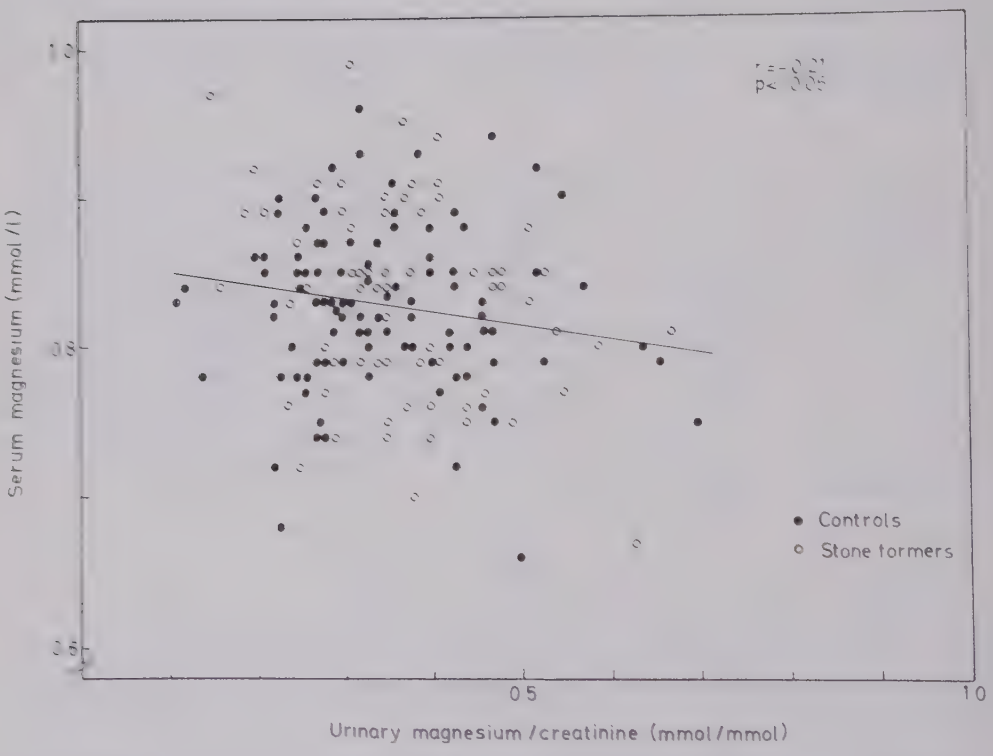

Fig. 7. The relation between serum magnesium concentration and the urinary magnesium excretion in healthy subjects and renal stone formers.

standardized, dietary intake of magnesium during the urine-sampling. The urinary excretion was inversely correlated to the serum magnesium values (Fig. 7), whereas these serum values were positively correlated to the intracellular concentration of magnesium in muscle cells (Fig. 8). The intracellular concentrations of magnesium and potassium were also closely correlated in all groups (II, III, IV).

The correlations between the different magnesium parameters in these groups were mostly weak, probably due to the narrow range of most magnesium values, but are in general in agreement with findings from other studies, utilizing similar methods, but also including subjects with disturbances of magnesium homeostasis and thus values outside the normal range (Alfrey et al., 1974). However, it was beyond the scope of the present investigation to confirm that the methods developed by others are reliable for the diagnosis of magnesium metabolism disorders. Rather, the purpose was to apply these, fairly established, techniques on different clinical materials.

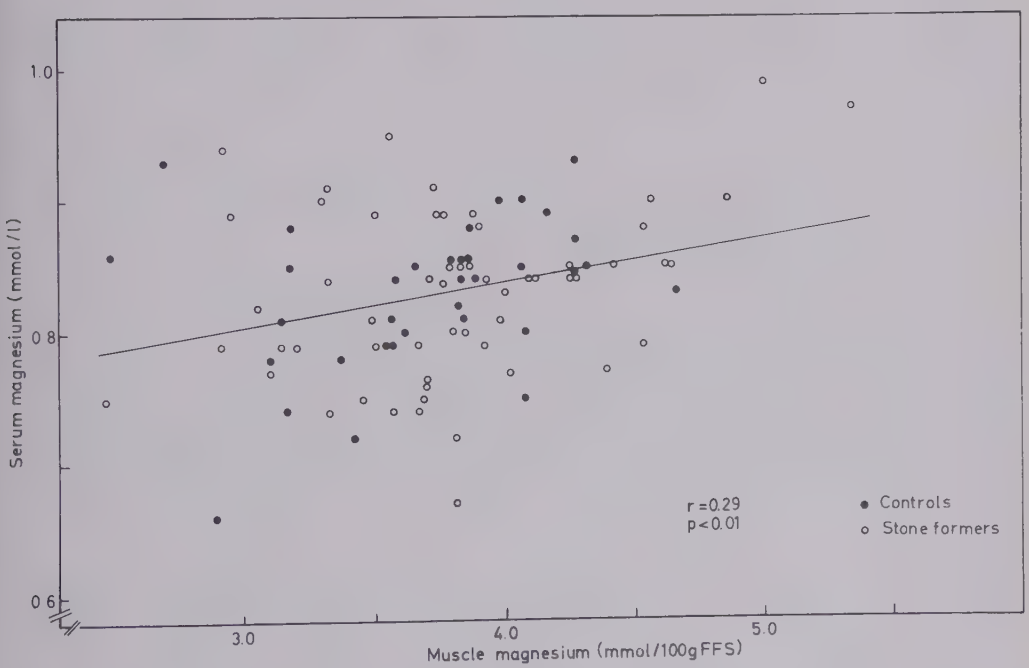

Fig. 8. The relation between the serum magnesium concentration and the muscle magnesium concentration in healthy subjects and renal stone formers.

Magnesium deficiency in HPT or renal stone disease ?

In order to disclose any possible magnesium deficiency in patients with HPT or renal stone disease, the patients with the lowest values for serum magnesium, urinary magnesium and muscle magnesium and the highest values for the magnesium retention were evaluated separately. However, whatever magnesium parameter was used, this analysis failed to display any difference in comparison with the remaining group or with the group of apparently healthy subjects (II, III, IV). In a similar way, stone patients with renal tubular defects were evaluated separately, but this analysis did not reveal any abnormalities of the magnesium metabolism associated with disturbed, renal tubular function (IV).

The muscle magnesium content was found to be unchanged, as compared with the pretreatment values, when 15 of the stone formers were re-investigated after two years' treatment with magnesium hydroxide.

In addition, both the magnesium retention and the muscle magnesium content were found to be unchanged in the patients with HPT, when they were re-investigated after parathyroidectomy (III).

In conclusion, with the methods utilized, magnesium deficiency of possible clinical importance appears to be uncommon in cases of in mild-to-moderate HPT and renal calcium stone disease.

Magnesium metabolism in primary hyperparathyroidism (III)

The magnesium metabolism was studied in 28 patients suffering from primary HPT before and three months after parathyroidectomy. The serum magnesium values were within the normal range in all patients, both before and after operation (Fig. 6). No correlation was observed between the serum magnesium and serum calcium concentrations before operation, in contrast to other reports (King & Stanbury, 1970; Sutton, 1970), probably due to the mild degree of hypercalcemia in our subjects. Preoperatively, the urinary magnesium excretion was slightly greater than in age- and sex-matched controls, whereas this difference was abolished after surgery (Fig. 3). The urinary

magnesium and urinary calcium excretions were positively correlated, both pre- and postoperatively (Fig. 4).

The fractional, gastrointestinal absorption of magnesium was reduced after parathyroidectomy. Similar results have earlier been obtained in conventional balance studies (Heaton & Pyrah, 1963: King & Stanbury, 1970). Both the intracellular magnesium concentration in muscle cells and the retention of magnesium after a loading test were normal before operation and remained unchanged after parathyroidectomy. It is concluded that magnesium deficiency of clinical importance seems to be uncommon in cases of mild-to-moderate, primary HPT.

No patient had an overt, hyperparathyroid, bone disease, but raised levels of alkaline phosphatases were encountered, in association with an increased retention of magnesium after the intravenously administered load. A postoperative drop in the alkaline-phosphatase activity was associated with a reduction of the serum magnesium concentration, suggesting an increased deposition of magnesium in the mineralizing bone. A similar drop in the serum magnesium concentration after parathyroidectomy has previously been reported only in patients with an overt bone disease (Hanna, North, MacIntyre & Fraser, 1961; Heaton & Pyrah, 1963; King & Stanbury, 1970).

Renal stone disease is common in cases of primary HPT, with a reported frequency of from 50 to 80 per cent (Hellström & Ivemark, 1962; Purnell, Smith, Scholz, Elveback & Arnaud, 1971; Werner, Hjern & Sjöberg, 1974). In this study, 14 out of 28 subjects presented a history of passing stones or had stones visualized on X-rays at admission. No special feature concerning the magnesium metabolism was observed in these patients in comparison with those without stone disease. In all patients, irrespective of renal stone disease, a preoperatively low, urinary magnesium/calcium ratio was normalized after surgery.

Magnesium metabolism in renal stone disease (IV)

Seventy consecutive patients suffering from renal stone disease and admitted to our stone clinic were evaluated with regard to their magnesium metabolism and compared with a control group matched for age and sex. There were no

differences in the serum and urinary magnesium values between the group of stone formers and the controls, although male stone formers had a higher output than the male controls. For all ages, male stone formers had a greater magnesium excretion than the female, stone-forming patients, but the age-dependent, magnesium excretion found in healthy subjects was not apparent in the stone formers. The excretion of calcium in the urine was higher in the stone formers than in the controls, and there was a positive correlation between the urinary excretions of magnesium and calcium, both in stone formers (Fig. 4) and in controls. A lower urinary magnesium/calcium ratio was found in the stone formers, mainly due to their greater urinary output of calcium. For both groups, this ratio declined with increasing calcium values, but the relationship was slightly shifted in the stone formers, who had a lower magnesium/calcium ratio than the controls for corresponding calcium values (Fig. 5).

An impaired, acidification capacity was found in 15 patients, and another six displayed tubular proteinuria. All patients with acidification defects had the incomplete type of renal tubular acidosis (RTA), 11 out of the 15 having proximal defects.

The intestinal absorption of magnesium in the stone formers was similar to that found in healthy subjects (Fig. 1). Compared with the controls, the stone formers also had similar values for the intracellular magnesium content and the magnesium retention of an intravenously given, magnesium load. Whichever method was used for assessing the magnesium metabolism, stone formers did not differ from the control group. Also, when the patients with renal tubular defects were evaluated separately, no differences appeared. Consequently, it seems unlikely that magnesium deficiency in man is of common, pathogenetic importance, as regards stone formation. However, a low urinary excretion of magnesium in relation to calcium may be of importance for the increased, stone-forming propensity.

Prophylactic treatment with magnesium of renal stone disease (V)

Prophylactic treatment with magnesium hydroxide (400 to 500 mg daily) was instituted in 56 of the 70 patients with renal stone disease who had previously been investigated with regard to their magnesium metabolism. There

was a slight increase in serum magnesium concentration during the first 6 months of treatment, after which the values returned to the pretreatment level. The urinary excretion increased by approximately 1.5 mmol/24 h during the first month, irrespective of the pretreatment excretion, and this larger excretion persisted during the entire follow-up period. No changes were observed in the serum and urinary calcium concentrations. Consequently, the urinary magnesium/calcium ratio increased and approached the value found in healthy subjects.

Most of the patients have now been under treatment for at least two years, and only 6 of them have experienced recurrence or formation of new stone. The mean stone-episode rate during treatment was 0.03 stones/year, to be

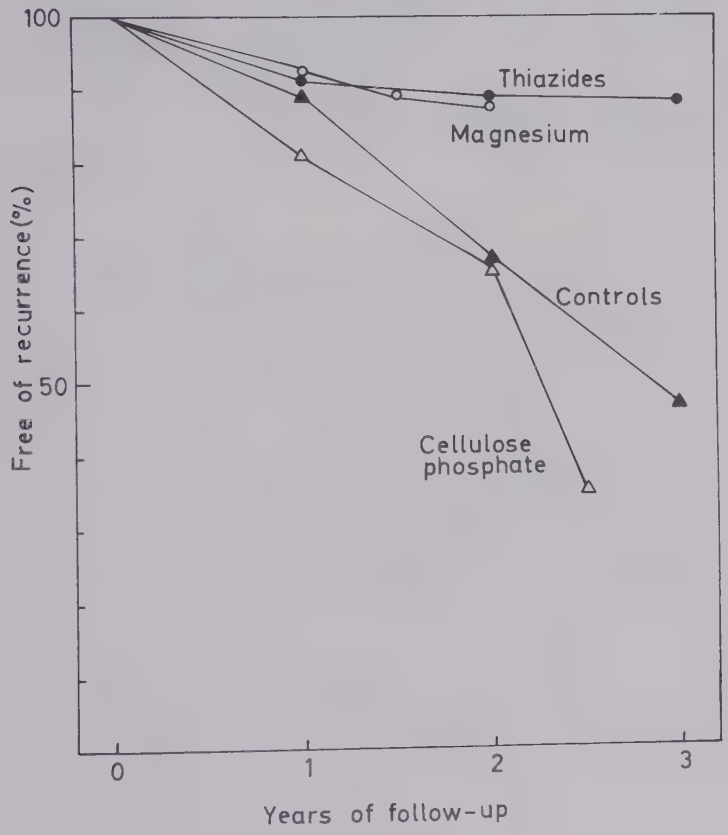

Fig. 9. The renal stone recurrence rates in subjects on prophylactic treatment with either thiazides, magnesium hydroxide or sodium-cellulose-phosphate in comparison with controls.

compared with 0.8 stones/year before treatment was instituted. For comparison, the natural history of the stone disease was followed at the stone clinic in 34 patients who were not on medical treatment, either because it was considered that their stone disease was not active enough for prophylactic therapy or, in some instances, because the patients themselves preferred to wait for some time. Of these 34 patients, 15 continued to form stones, i.e. they had a total recurrence rate after two years' observation of 44 per cent. Thus, in comparison, treatment with magnesium hydroxide appeared to reduce the recurrence rate. This beneficial effect is also in agreement with earlier reports (Moore & Bunce, 1964; Melnick et al., 1971; Prien & Gershoff, 1974). The results are also comparable with our earlier experiences, as well as those of other authors, with thiazides as prophylactic treatment against renal stones (Coe, 1977; Yendt & Cohanim, 1978; Backman, Danielson, Johansson, Ljunghall & Wikström, 1979 a), whereas both appear to be more effective than cellulose phosphate (Backman, Danielson, Johansson, Ljunghall & Wikström, 1979 b) (Fig. 9). Besides minor gastrointestinal discomfort, no adverse effects were observed during the treatment.

GENERAL SUMMARY

1. The magnesium metabolism was studied in apparently healthy subjects and in patients suffering from primary hyperparathyroidism (HPT) or renal stone disease.

2. A technique utilizing the radionuclide ^{28}Mg was developed to assess the gastrointestinal absorption of magnesium. External, photon detectors were used to measure the uptake of the radionuclide in the forearm after both intravenous and oral administration (I).

3. The time derivation of the oral uptake curve indicated a fairly uniform absorption of ^{28}Mg throughout the small intestine (I). The fractional, intestinal absorption in healthy subjects was 42 $\pm$ 7 % (mean $\pm$ SD) (I, II). The absorption decreased with age.

4. The serum magnesium concentration was remarkably constant in all the groups studied (II, III, IV).

5. The basal, urinary excretion of magnesium was greater in males than in females at all ages (II, IV) and decreased with age (II). The urinary concentrations of magnesium and calcium were closely correlated in all the groups studied (II, III, IV).

6. The intracellular magnesium content was determined, as an indicator of the magnesium body stores, in muscle specimens obtained by percutaneous biopsies (II, III, IV, V). The magnesium retention was calculated from the urinary excretion after a dose of magnesium had been given intravenously (II, III, IV, V).

7. As assessed by the methods utilized, magnesium deficiency was not a common feature in cases of renal stone disease or mild-to-moderate primary HPT (III, IV, V).

8. The fractional, intestinal absorption of magnesium in patients with HPT decreased after parathyroidectomy. No patient with HPT had an overt, hyperparathyroid, bone disease. The magnesium retention was positively

correlated to the serum alkaline-phosphatase activity before operation. A postoperative drop in the serum alkaline-phosphatase was associated with a decrease in the serum magnesium concentration. Serum and urinary magnesium levels were little affected by parathyroidectomy (III).

9. No significant deviations in the magnesium metabolism were observed in patients with renal stone formation (IV).

10. Prophylactic treatment with magnesium hydroxide of cases of renal stone disease was associated with an increased, urinary magnesium excretion and a beneficial effect on the stone-recurrence rate, in comparison with a control group. The side effects were few (V).

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MAGNESIUM METABOLISM IN HEALTHY SUBJECTS

by

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SUMMARY

Magnesium metabolism was studied in 106 apparently healthy subjects aged between 15 and 80 years. The serum magnesium values had a gaussian distribution with a narrow range and with no age or sex dependence. The basal, urinary magnesium excretion was greater in men than in women at all ages and decreased with age. The magnesium and calcium excretions were closely correlated in both sexes. The fractional, gastrointestinal absorption of magnesium, assessed by a newly developed technique using the radionuclide ^{28}Mg, compared favourably with that in earlier balance and tracer studies. The intracellular magnesium content, estimated in muscle specimens obtained by percutaneous biopsies, was not correlated to age or sex. A magnesium-loading test was performed, and the normal range for magnesium retention was calculated. No correlation was demonstrated between different magnesium parameters within the normal range in these subjects.

INTRODUCTION

Magnesium is the major, divalent, intracellular cation and participates in a wide variety of fundamental biological reactions. The adult human body contains about 1 mole magnesium, approximately 60 % in the skeleton and the rest in the soft tissues, except for a small fraction (1 %) located extracellularly (Aikawa, 1976).

The fractional, magnesium absorption in the intestine is mainly influenced by the dietary load presented to the intestinal mucosa (Graham, Caesar & Burgen, 1960). During reduced intake or decreased absorption of magnesium, there is a potent, renal conservation with an almost complete, tubular reabsorption of magnesium (Fitzgerald & Fourman, 1956; Dunn & Walser, 1966; Shils, 1969). However, no known homeostatic system which mobilizes magnesium from the body stores operates to maintain the plasma levels, and magnesium deficiency may exist with low, normal or elevated magnesium levels in the blood (Fitzgerald & Fourman, 1956; Caddell, 1969; Lim, Dong & Khoo, 1969 a). Thus, it appears that the intestinal absorption and the urinary excretion of magnesium are the main factors regulating the serum magnesium levels (MacIntyre & Robinson, 1969).

Technical difficulties in determining the cellular content of magnesium in different tissues have previously compelled to rely on the levels of magnesium in the blood as an index of magnesium deficiency, but it is likely that the diagnosis of magnesium deficiency also requires assessment of the content in other body stores (Lim, Jacob, Dong & Khoo, 1969 b). As magnesium in the muscles appears to be the most readily accessible reservoir buffering losses of magnesium from the body, an early sign of magnesium deficiency would be reflected in the muscle content (Lim et al., 1969 b).

Another way of assessing magnesium requirements may be made indirectly from calculations of the retention of magnesium loading, since the need for magnesium is increased in a state of deficiency and more of the administered load is then retained in the body (Thorén, 1963; Jones & Fourman, 1966).

The investigation was performed in order to establish the normal range for the following magnesium parameters in healthy subjects: serum and urinary concentration, intracellular content, intestinal absorption, and retention of an intravenously (i.v.) administered magnesium load. The distribution of these parameters was analyzed with relation to sex and age and the interrelationships between the different parameters were studied.

SUBJECTS

This study of magnesium metabolism in 106 apparently healthy subjects was performed on individuals from three groups:

1. Sixty-five apparently healthy subjects were recruited from a general health survey in such a fashion that, for each decade, 10 individuals (five men and five women) between the ages of 15 and 85 were investigated, in order to evaluate the possible variations of magnesium values in serum and urine according to sex and age.

2. Thirteen medical students and 16 members of the medical staff were studied more extensively, including an intravenous magnesium loading test and a muscle biopsy, and in 16 males an assessment of the gastrointestinal absorption of ^{28}Mg.

3. Twelve patients admitted to the hospital for the evaluation of various symptoms, mainly because of suspicion of a primary hyperparathyroidism, were also included, because a detailed evaluation failed to disclose any disturbance in calcium metabolism. These patients were studied as in-patients in a regular, medical ward, while receiving the hospital diet.

   All the other subjects had a diet of their own choice and were not restricted in their daily living. No subject had any regular medication and in particular no history of renal stone disease. All the subjects were informed of the purpose of the study and their consent was obtained verbally. As separate analysis of each group failed to disclose any differences between the groups, all the subjects were finally pooled together.

Serum magnesium and serum calcium concentrations were determined by atomic absorption in a single blood sample after an overnight fast by all subjects.

Urinary excretion of electrolytes was calculated from a single, 24-hour, urine collection in plastic bottles without additives. The clearance of creatinine was simultaneously calculated in order to disclose major sampling errors. Magnesium and calcium were determined by atomic absorption.

The gastrointestinal absorption of magnesium was estimated by a technique utilizing the radionuclide ^{28}Mg, described elsewhere (Danielson, Johansson, Jung, Ljunghall, Lundqvist & Malmborg, 1978). The radionuclide was administered both orally, together with a standard breakfast, and after an interval of 2 - 7 days also intravenously. The radioactivity was measured over the forearm and the fractional, gastrointestinal absorption of magnesium was calculated as the quotient between the activity values obtained after the oral and the intravenous administration. The study was performed in 16 males between 22 and 40 years of age. The coefficient of variation was calculated as 19 %.

The intracellular content of magnesium and potassium in muscle cells was determined by a technique according to Bergström (1962). A piece of muscle with a wet weight of 15 - 20 mg was obtained with a specially designed, biopsy needle from the lateral portion of the lateral quadriceps muscle about 15 - 20 cm above the knee. After careful elimination under the microscope of fat, connective tissue and excess blood, the specimen was dried in an oven in 90^{o}C to a constant weight. Neutral fat was then extracted with petroleum ether, after which the biopsy was dried again. This fat-free, solid weight (FFS) was used as the reference value. In order to dissolve the dried and weighed biopsy specimen, it was transferred to a test tube with 1 ml of 1-molar nitric acid. After another 12 hours, a diluting fluid with an excess of iron and phosphorus was added, in order to keep the interference from these ions constant (Bergström, 1973). An atomic absorption spectrophotometer (Perkin Elmer 303) was used for the determination of the magnesium and potassium contents of the sample. The coefficients of variation were 3.3 % and 2.6 %, respectively. Adequate biopsies were obtained from 35 subjects.

A magnesium-loading test was performed (Thorén, 1963), in which 10 mmoles magnesium, in the form of magnesium sulphate, were given i.v. during three hours as an infusion in a glucose-fructose solution (Inverdex®, 500 ml). Urine was collected for 24 hours from the start of the infusion. This test was performed in 34 subjects. The patients were not restricted in their dietary intake during this test or during the collection of the basal urine. The coefficient of variation in five subjects was 8 %.

Magnesium retention was calculated from the value of magnesium excretion obtained after the loading test. This calculation also took into account the patient's basal, urinary output of magnesium, i.e. the magnesium contributed through the intestinal absorption of dietary magnesium, which was believed to be constant during the days of study. The difference between these values was supposed to reflect the amount of the infused magnesium excreted in the urine after loading. The retention was thus calculated as the difference between the infused and the adjusted excreted amount as a percentage of the infused amount. For example, a patient with a basal magnesium excretion of 6 mmoles, who, after loading with 10 mmoles, excreted 12 mmoles magnesium was assigned the magnesium retention of 40 % ($\frac{10 - (12 - 6)}{10} \times 100$). Another way of expressing the retention was by calculating the total load during the study, i.e. the sum of the infused amount and the amount corresponding to the basal output. The difference between this total load and the amount excreted after the loading was expressed as a percentage of the total load. This method of calculation yielded in the same case a retention of 25 % ($\frac{(10 + 6) - 12}{10 + 6} \times 100$). In the following text, the retention has been calculated by this latter method from the total load.

Chemical analyses were performed as routine laboratory tests at the Department of Clinical Chemistry.

Conventional statistical methods were used.

RESULTS

The distribution of the 106 apparently healthy subjects with respect to age and sex is shown in Fig. 1. Table I summarizes various magnesium data.

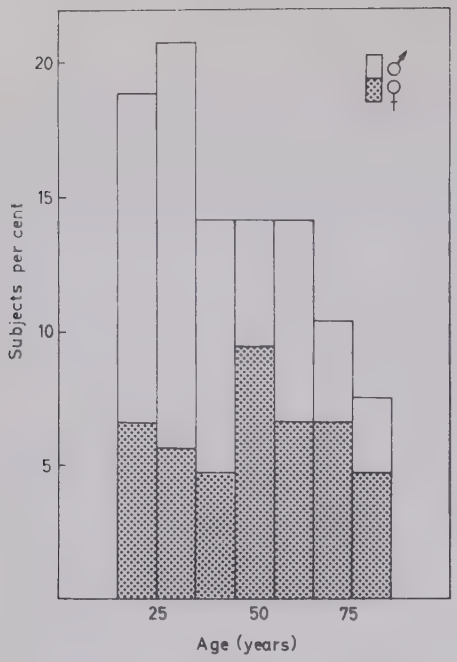
Table I. Magnesium data in 106 apparently healthy subjects. The figures within parentheses denote the numbers of subjects studied. Mean ± S.D.

	Women (n = 47)		Men (n = 59)		Total range
Serum magnesium (mmol/l)	0.82 ± 0.06		0.83 ± 0.05		0.66 - 0.96
Urinary magnesium (mmol/24 h)	3.6 ± 1.4		4.8 ± 1.5***		1.3 - 10.6
Urinary magnesium/calcium	1.1 ± 0.6		1.1 ± 0.7		0.2 - 4.1
Urinary magnesium after Mg-load (mmol/24 h)	11.0 ± 2.1	(12)	12.7 ± 2.2*	(22)	7.6 - 17.3
Magnesium retention[1] (%)	23 ± 11	(12)	18 ± 11	(22)	0 - 38
Magnesium retention[2] (%)	31 ± 15	(12)	28 ± 18	(22)	0 - 60
Magnesium absorption (%)	-		42 ± 7	(16)	32 - 56
Intracellular magnesium (mmol/100 g FFS)	3.8 ± 0.5	(9)	3.7 ± 0.5	(26)	2.5 - 4.7

*) $p < 0.05$; ***) $p < 0.001$, compared with women.

1) Retained amount in relation to i.v. magnesium load.

2) Retained amount in relation to total magnesium load. See text for detailed explanation.



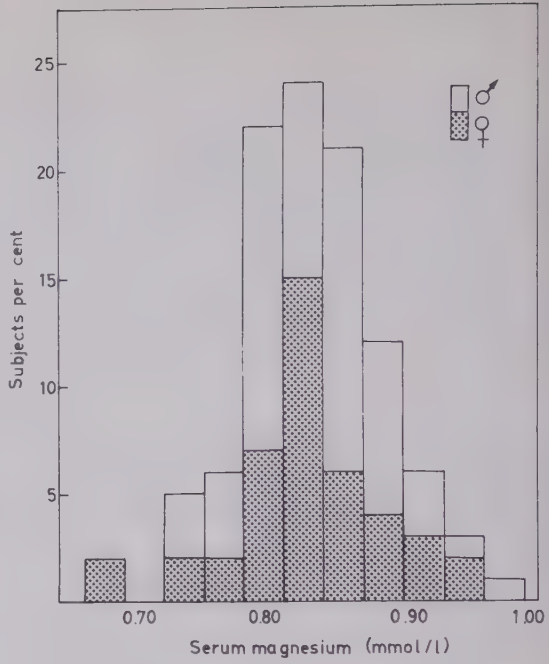


Fig. 1. Frequency distribution of the 106 apparently healthy men and women.

Fig. 2. Frequency distribution of serum magnesium in apparently healthy men and women.

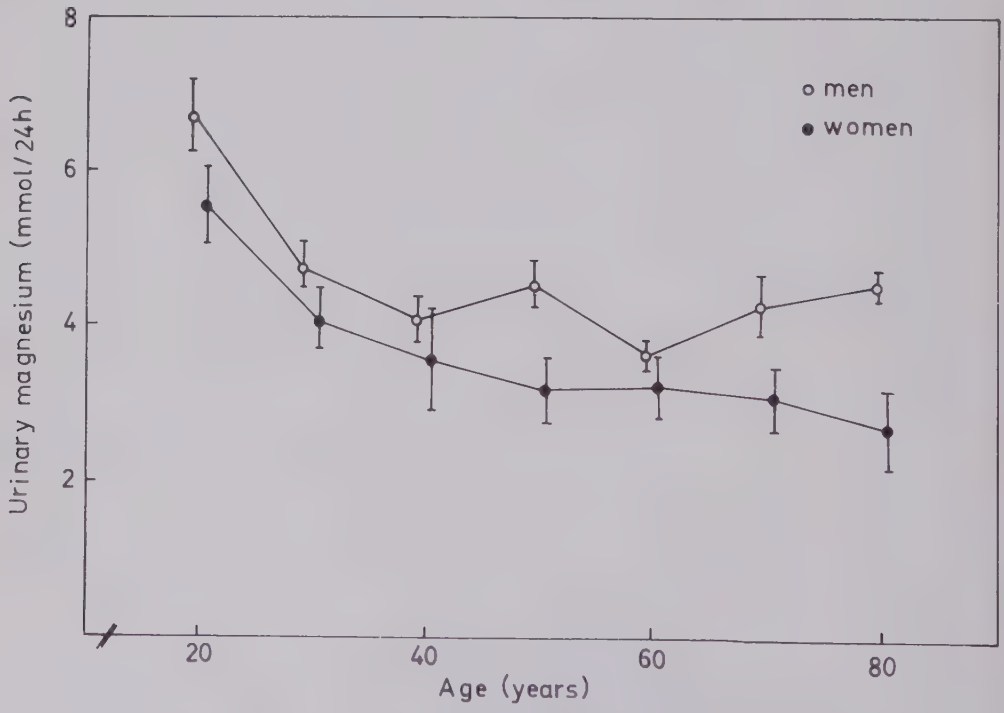


Fig. 3. Variation with age of the mean (± SEM), daily, urinary magnesium excretion in apparently healthy men and women.

Serum magnesium concentration

Serum magnesium showed a gaussian distribution (Fig. 2) between 0.66 and 0.96 mmol/l. There were no differences in the mean values between males and females, nor were there any variations according to age. The serum magnesium concentration was not correlated to the serum calcium concentration ($\underline{r}$ = 0.17) or to the serum albumin concentration ($\underline{r}$ = - 0.01).

Urinary magnesium excretion

The average, urinary magnesium excretion was higher in men than in women (Table I) at all ages with a reduction in output with increasing age for both sexes (Fig. 3). The difference between the sexes was abolished when the values were adjusted for body weight or urinary creatinine excretion

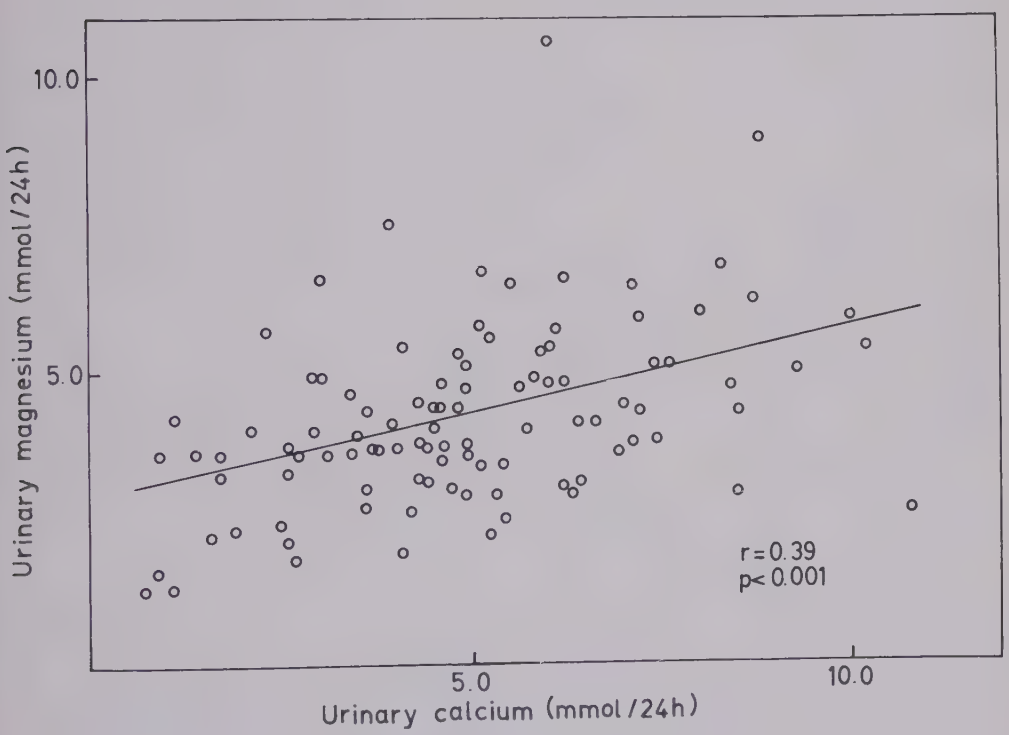


Fig. 4. Relation between urinary magnesium and calcium excretion in apparently healthy subjects.

(Table II). Day-to-day variations were studied in five healthy males and showed a coefficient of variation of 13 %.

Relation between urinary magnesium and calcium. Magnesium/calcium ratio

There was a positive correlation between the urinary magnesium and calcium (Fig. 4), which persisted whatever the mode of expression of the urinary excretion (i.e. in absolute amounts or related to urinary creatinine, clearance of creatinine, body weight or urine volume). The relationship was similar in men and women, although males had higher values for magnesium as well as for calcium excretion. There were no differences in the ratio of urinary magnesium to urinary calcium with respect to age or sex. The coefficient of variation for day-to-day collections was 16 %. The variation of the magnesium/calcium quotient with regard to the urinary output of each ion is

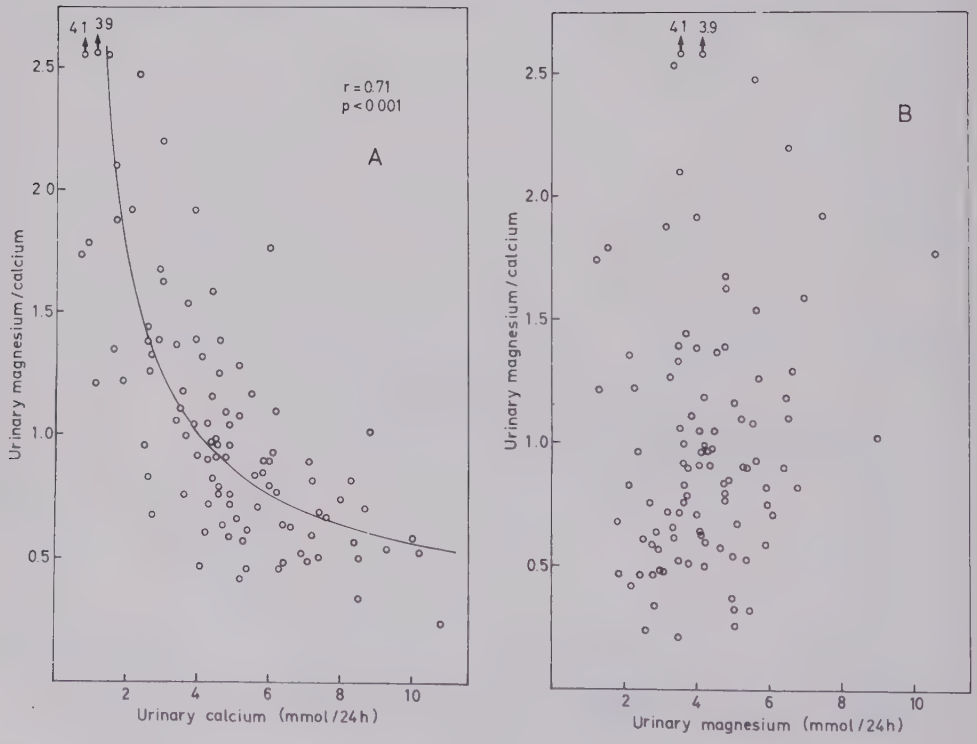


Fig. 5. Relation between the urinary magnesium/calcium ratio and the urinary calcium (A) and magnesium (B) excretions in apparently healthy subjects.

Table II. Urinary magnesium excretion in normal men and women.

	Women (n = 47)		Men (n = 59)	
	Mean ± S.D.	Coefficient of variation %	Mean ± S.D.	Coefficient of variation %
Urinary magnesium (mmol/24 h)	3.6 ± 1.4	39	4.8 ± 1.5***	31
Urinary magnesium/creatinine (mmol/mmol)	0.4 ± 0.1	32	0.3 ± 0.1	30
Urinary magnesium/creatinine clearance (mmol/ml/min)	0.04 ± 0.02	45	0.04 ± 0.01	28
Urinary magnesium/urine volume (mmol/l)	3.2 ± 1.7	53	3.5 ± 1.4	40
Urinary magnesium/body weight (mmol/kg)	0.06 ± 0.02	32	0.05 ± 0.02	29

***) $p < 0.001$, compared with women.

demonstrated in Fig. 5. This analysis showed that, while increasing values for urinary calcium were correlated to decreasing values of the ratio according to a hyperbolic function, no such consistent relation could be obtained for variations in the urinary magnesium.

Intestinal absorption of ^{28}Mg

The gastrointestinal absorption of magnesium, assessed by a radionuclide technique, ranged between 32 and 56 % (Fig. 6), with a mean value of 42 $\pm$ 7 % ($\pm$ S.D.).

Muscle magnesium concentration

The intracellular concentration of magnesium in muscle cells varied between 2.5 and 4.7 mmol/100 g FFS (Fig. 7) and was not related to age or sex. There was a close relationship between the muscle contents of magnesium and potassium (Fig. 8).

Urinary magnesium excretion after magnesium loading. Magnesium retention

The urinary excretion after loading with 10 mmoles magnesium was, on the average, 12.1 $\pm$ 2.3 (S.D.) mmoles per 24 hours. The after-load excretion was closely related to the basal, urinary magnesium (Fig. 9). The distribution for the magnesium retention values, calculated from the total load, is shown in Fig. 10. The serum magnesium concentration increased, on the average, by 0.4 mmol/l during the loading, with a normalization within one hour after the infusion.

Relation between different magnesium parameters

No correlation was observed between the values for serum magnesium, urinary magnesium (basal or after magnesium loading), magnesium retention, magnesium absorption or intracellular magnesium content within the normal range demonstrated in these subjects. In addition, the patients with the lowest values for serum magnesium, urinary magnesium, magnesium absorption and muscle magnesium or the highest values for magnesium retention did not show any divergence in the other magnesium parameters studied.

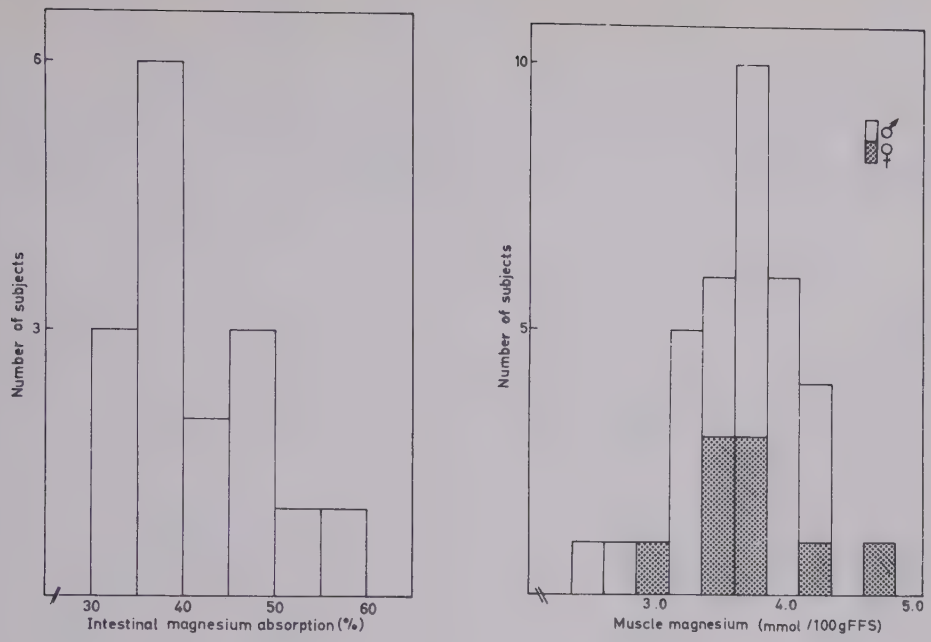


Fig. 6. Distribution of the fractional, intestinal, magnesium absorption in 16 apparently healthy men.

Fig. 7. Distribution of the muscle magnesium content in 35 apparently healthy men and women.

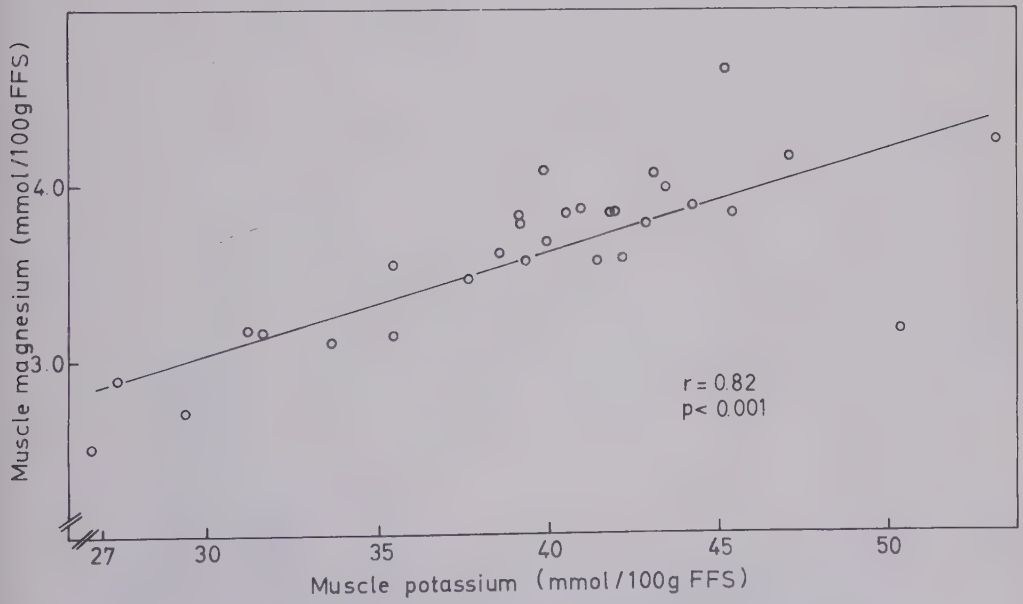


Fig. 8. Relation between the muscle magnesium and potassium content in apparently healthy subjects.

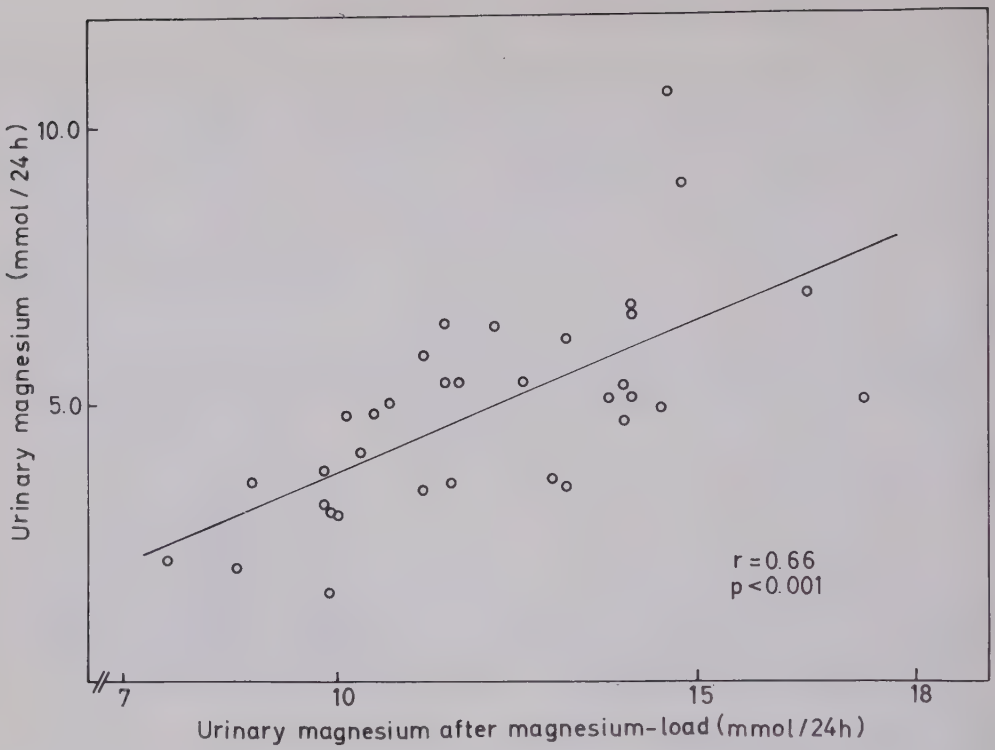


Fig. 9. Relation between the basal urinary excretion of magnesium and the urinary excretion of magnesium after an intravenously administered magnesium load in 34 apparently healthy subjects.

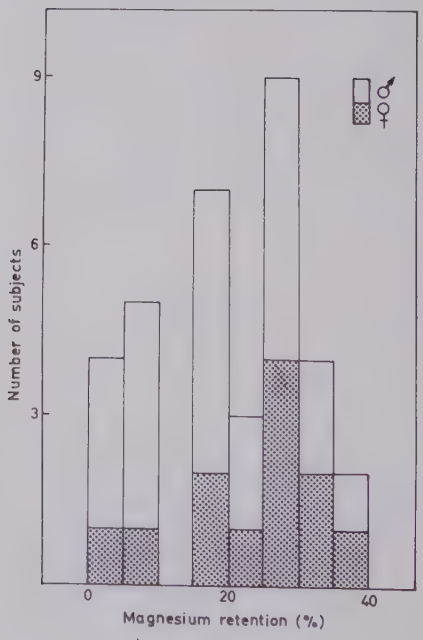


Fig. 10. Distribution of the retention of an intravenously administered magnesium load in 34 apparently healthy men and women.

DISCUSSION

The purpose of this study was to establish a frame of reference for different aspects of magnesium homeostasis in apparently healthy subjects as a basis for the evaluation of magnesium metabolism in various clinical disorders.

Serum magnesium concentration

Although no known homeostatic system operates to regulate the serum magnesium levels, the values are remarkably constant between individuals; in the present study with a variation of 7 % of the mean value and also within individuals with a precision of 0.6 % (Petersen, Schroll, Christiansen & Transbøl, 1977). The mean values and the distribution of the serum magnesium concentrations, as well as the lack of age and sex dependence in our subjects, agree fairly well with previous reports in the literature (Briscoe & Ragan, 1967; Jackson & Meier, 1968; Keating, Jones, Elveback & Randall, 1969; Christiansen & Transbøl, 1978). In the plasma, 20 - 30 % of the magnesium is bound to proteins, the major part to albumin, while the rest is present in a diffusible form with the major fraction as free, ionized magnesium (Massry & Seelig, 1977; Danielson, Pallin & Sohtell, 1979). A weak correlation has sometimes been found between the serum values of magnesium and albumin (Keating et al., 1969) and this association is likely to be the cause of the correlation shown between the serum values for magnesium and calcium (Briscoe & Ragan, 1967). However, this correlation was abolished when albumin- och protein-correlated values were used, which suggests a false relationship because of changes depending on body posture or deviation in serum proteins due to venous stasis during the blood-sampling (Christiansen & Transbøl, 1978). In the present study, no such correlation could be found, probably due to the narrow range of both the serum albumin and serum magnesium concentrations in these healthy subjects.

Urinary magnesium excretion

The average urinary excretion of magnesium was greater in healthy men than in healthy women at all ages and the values decreased with age in both sexes. Correction for body weight did not reduce this age dependence in

either sex but eliminated the differences between the sexes. On the other hand, correction for lean body mass by the use of urinary creatinine (Muldowney, Crooks & Bluhm, 1957), eliminated both the age and sex dependence in urinary magnesium output. The different degrees of correction produced by these two ratios could probably be accounted for by a continuous fall in the creatinine/body weight ratio with increasing age, presumably as loss of muscle tissue in later life is partially offset by a gain in fat (Robertson, 1976). There appears to be a slight reduction of the fractional absorption with age (Johansson, 1979) which, however, not was apparent within the narrow age range in the present study. This reduction with age, possibly together with a lower dietary intake, is likely to explain the reduced magnesium excretion in the higher age groups.

Further evaluation of the various ways of expressing magnesium output showed that the coefficient of variation was slightly reduced when the excretion was related to urinary creatinine. This probably reflected not only the dependence on lean body mass, but also the correction which creatinine made for incomplete collections of urine. However, the differences between the various ways of expressing urinary magnesium were small and it seems that, for practical reasons, correction of urinary electrolytes for the simultaneously obtained creatinine offers no particular advantages. However, it is important to consider the variations in magnesium excretion with age and sex, when collecting control materials for clinical purposes.

Intestinal absorption

Gastrointestinal absorption of magnesium has generally been assessed by the use of conventional balance studies. Wilkinson (1976) reviewed the literature and found an average for net absorption of 35 - 40 %. Few previous studies have assessed magnesium absorption by isotope techniques. Avioli & Berman (1966) found that the true absorption in healthy volunteers averaged 46 % (range 38 - 56 %) and similar absorption values, although based on studies of only a few individuals, are also reported by Graham et al. (1960) and Schwartz, Spencer & Wentworth (1978). Thus, our range of 32 % to 56 % for the true absorption of magnesium in healthy subjects, with a mean value of 42 %, compares favourably with both previous balance and tracer studies.

Over a wide range of intake, there exists a linear relationship between the intake of magnesium and the amount absorbed, i.e. the absorbed fraction is constant within the normal range of dietary magnesium (King & Stanbury, 1970). The urinary magnesium output is thus mainly dependent on the magnesium intake and presumably individual variations in dietary magnesium among our volunteers account for the lack of correlation between the fractional absorption determined and the urinary magnesium output.

Interaction between magnesium and calcium

The magnesium transport in the intestine involves two processes, both a diffusion component and a saturable component, in a similar way to calcium (Wilkinson, 1976). The saturable component appears to be a facilitated-diffusion process for magnesium, in contrast to the active transport found for calcium (Care & van't Klooster, 1965; O'Donnel & Smith, 1973). Magnesium and calcium are probably partly absorbed by a common pathway (Alcock & MacIntyre, 1962; Heaton, Hodgkinson & Rose, 1964; Clarkson, Warren, MacDonald & de Wardener, 1967) with a simultaneous competition between the two ions (Heaton et al., 1964). In general, however, there is a correlation, although with a wide spread, between magnesium and calcium absorption in balance studies. This relationship may be due to dietary or digestive factors, but also to the fact that vitamin D and parathyroid hormone appear to have similar effects on calcium and magnesium absorption (Wilkinson, 1976; Levine, Brautbar, Lee, Walling, Kurokawa, Kleeman & Coburn, 1979).

Also in the renal tubules, magnesium and calcium appear to share a common transport system for reabsorption (Massry & Coburn, 1973). This results in an increased magnesium excretion, when the filtered load of calcium is increased, provided the parathyroid hormone secretion is unchanged.

These common pathways for magnesium and calcium in the intestine and the kidney are likely to be a main cause of the positive relation between the urinary excretions found both during short-term studies (Massry & Coburn, 1973) and, as in the present study, in the steady state (King & Stanbury, 1970; Welshman & McGeown, 1975; Ljunghall & Waern, 1977).

Urinary magnesium/calcium ratio

The urinary magnesium/calcium ratio was calculated in this study, mainly because it has been found to be a valuable index of the propensity to calcium-stone formation (Oreopoulos, Soyannwo & McGeown, 1968; Welshman & McGeown, 1975; Ljunghall & Waern, 1977; Tiselius, Almgård, Larsson & Sörbo, 1978). A high urinary calcium excretion increases the risk of calcium crystal precipitation and growth (Robertson, Peacock, Heyburn, Marshall & Clark, 1978), whereas an increased amount of magnesium, at least *in vitro*, increases the solubility of calcium oxalate (Desmars & Tawashi, 1973). From previous reports concerned with calculations of this ratio, it has not always been possible to evaluate whether variations of the ratio of magnesium to calcium are due primarily to changes in urinary magnesium, urinary calcium or both ions simultaneously (Oreopoulos et al., 1968). As the values for urinary magnesium and calcium are linearly related, the corresponding graph between the quotient and the urinary calcium is best described by a hyperbolic curve (Fig. 5). The ratio was, however, significantly correlated to the calcium (r = 0.71) but not to the magnesium excretion (r = -0.08), probably because of the broader range of urinary calcium.

Intracellular magnesium concentration

Bone contains about 60 % of the total body magnesium, the remainder being almost equally distributed between muscles and non-muscular, soft tissues (Aikawa, 1976). From isotope studies, it has been concluded that muscle magnesium is a readily accessible reservoir that buffers losses of magnesium from the body, while bone magnesium is relatively inaccessible, unless a state of prolonged depletion is present (Lim et al., 1969 b). Moreover, the relative ease of obtaining muscle specimens, compared with bone biopsies, makes the estimation of muscle magnesium a more convenient and reliable method. Since the ratio of intracellular to extracellular magnesium is about 40 to 1 (Widdowson, McCance & Spray, 1951) it is also obvious that the estimation of muscle magnesium is essentially one of intracellular magnesium. During dietary magnesium restriction or in states of magnesium malabsorption, decreased muscle magnesium concentrations have been demonstrated in combination with low or normal serum levels (Caddell, 1969; Lim, et al., 1969 b; Lim & Jacob, 1972), though others have not been able to confirm these fin-

dings (Dunn & Walser, 1966; Alfrey, Miller & Butkus, 1974). In our normal subjects, no sex and age differences could be demonstrated and the mean value and range for muscle magnesium compared fairly well with those in the literature (Lim et al., 1969 b; Bergström et al., 1973).

Magnesium retention

As a complement to the direct measurement of magnesium concentrations in various body compartments, an indirect way of assessing magnesium needs is provided by the calculation of retention after magnesium loading (Jones & Fourman, 1966). Experimental studies have demonstrated a low, faecal elimination of endogenous ^{28}Mg, 1 - 2 % (Avioli & Berman, 1966), and a high tubular reabsorption process working close to its maximum under basal conditions (Massry & Coburn, 1973). Aikawa (1976) also demonstrated a high retention of ^{28}Mg when given intravenously with stable magnesium and a simultaneous, urinary excretion of magnesium after the load equivalent to the given amount. This indicates a high degree of retention of the load, but a compensatory, renal excretion of magnesium from body stores of other ions than those given. Thus, most of the i.v. given magnesium will be excreted in the urine in healthy subjects with normal renal functions (Thorén, 1963; Caddell, Ratananon & Trangratapit, 1973).

Generally, only the 24-hour, urinary excretion of magnesium after the load has been determined as an index of magnesium retention (Thorén, 1963; Jones & Fourman, 1966). However, as Caddell et al. (1973) emphasized, this calculation is sometimes invalid, unless the post-load excretion is corrected for the basal output, because of the close relationship between these two parameters (Fig. 9). This correction may be made either by simply subtracting the basal output from the given amount and relating this value to the i.v. given load. However, during the day of study, also some magnesium is contributed through intestinal absorption. As the amount absorbed in individuals in magnesium balance is closely correlated to the amount excreted, the total absorbed magnesium may for practical purposes be considered equal to the urinary output. Thus, the total magnesium load given may be approximated as the i.v. given amount plus the value obtained from the urinary, basal output. The retention is then calculated as the difference between the total load and the excreted amount after loading expressed in relation to the total magnesium load.

It seems reasonable to suggest that our healthy volunteers were in magnesium balance and thus a small variation of the retention should be expected. If no adjustment was made for the basal urinary output, the post-load excretion showed a great variation, but when a correction was made in either of the two ways described, and in particular when related to the total load, the range of retention was considerably narrowed. Also, for this reason, it seems appropriate to consider also the basal magnesium excretion when the retention is calculated and to correlate it to the total load.

Estimation of body stores

In this study, several different parameters have been investigated, in order to provide a basis for the detection of magnesium deficiency in clinical practice. Within the normal range in these healthy subjects, there were no correlations between the studied parameters, nor was any consistent pattern observed for the subjects with the lowest values. Such a correlation is also unlikely to exist, unless values outside the normal range are included. When similar methods were applied to the investigation of conditions associated with disturbed, magnesium homeostasis (Alfrey et al., 1974), there was also a fairly close correlation between the serum and the muscle or bone magnesium, suggesting that either of these would be helpful in the diagnosis of magnesium deficiency. However, it is likely that diagnosis of a possible magnesium deficiency requires determinations not only of serum and urinary levels but also of the magnesium concentration in other body stores.

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